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REVIEWS

BIOLOGICAL LEACHING OF SHALES - A REVIEW

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An overview of microbial technology for sulfur removal from shales is presented in this paper. Sulfur removal from oil shales through bioprocessing can substantially reduce SO_2 emissions from thermal power plants that combust oil shales for energy production. Iron sulfides constitute the most significant inorganic sulfur fraction in shales. There are several Fe- and S-oxidizing microorganisms that have potential for oxidizing iron sulfides to soluble products in acid sulfate-containing solutions.

Environmental factors that influence the activities of these organisms are discussed. The organic sulfur compounds in shale matrix are variable and complex. Microbiological transformations of the organic sulfur fraction are poorly understood and it is not clear whether they are amenable to shale bioprocessing.

The paper summarizes previously published experimental studies where shales were contacted with bacteria for iron sulfide oxidation. Shales constitute a complex system of study because they contain several phases such as clay minerals, sulfides, organic matter, and carbonates that interact with each other and with microorganisms.

Introduction

The purpose of this paper is to present an overview of microbial technology for sulfur removal from shales. Shales are fine-grained, sedimentary mixtures of clay minerals (e.g., illite, montmorillonite), quartz, and micas that have been usually formed by consolidation of mud,

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Table 1. **Mineralogical Characteristics of Estonian Shale Samples Used in Preliminary Leaching Experiments**

Sample	Qualitative mineralogical composition*
Black shale from Toolse phosphorite deposit	Quartz, K-feldspar (orthoclase), pyrite, illite, kaolinite
Kerogen-rich flotation concentrate of Toolse black shale	Quartz, K-feldspar (orthoclase), pyrite, illite, kaolinite
Pyrite-rich flotation concentrate of Toolse black shale	Quartz, K-feldspar (orthoclase), pyrite, illite, kaolinite, marcasite
Ash residue of Toolse black shale	Quartz, K-feldspar (orthoclase), hematite, illite, gypsum
Black shale from Maardu phosphorite deposit	Quartz, K-feldspar (orthoclase), pyrite, illite
Oil shale from Ida-Virumaa	Quartz, dolomite, calcite, pyrite

* Major mineral phases identified by X-ray diffraction; data pooled from [51, 58] and courtesy of Dr. Martti K. Lehtinen, Geological Museum, University of Helsinki, Finland.

clay, or silt. Several types of shales exist depending on the conditions of formation and the associated materials. Some shale deposits may have economic importance because of kerogen content; environmental concerns may also be involved with their commercial mining because of water pollution by leachates containing elevated levels of metals. Table 1 lists major minerals identified in several Estonian shale samples.

Oil-containing shales constitute a significant resource of fossil fuel in many countries, including Estonia where energy production is reliant on using domestic oil shales in thermal power plants. Fossil fuel combustion (oil shale, coal) is a major source of anthropogenic SO_2 emission as well as of other air pollutants such as fly ash, CO_2, and NO_x. SO_2 emissions due to oil shale combustion can be reduced with installation and operation of air filters for cleaning flue gases, or with pre-combustion treatment with chemicals or with microorganisms. Biological removal of sulfur (Table 2) relies on the microbiological oxidation of S-compounds to water-soluble products, principally to sulfates that can be subsequently washed off before combustion. The microbial technology minimizes the loss of British

Table 2. **Categorical Representation of Microbiological Removal of Sulfur from Fossil Fuel**

Sulfur fraction in fossil fuel	Main groups of compounds	Microbes
Inorganic S	Fe-sulfides	Acidophilic Fe- and S-oxidizing bacteria, oxidation to water-soluble products (Fe^{3+} and SO_4^{2-})
Organic S	Organic thiols, sulfides, and thiophenes	Heterophic bacteria, partial degradation to various non-polar and water-soluble products

Thermal Unit (BTU) content during sulfur removal if the organic carbon matrix is not destroyed.

In principle, the biological treatment also decreases the ash content due to the leaching of metals in acid solutions and it may be economically competitive with the chemical and physical methods of S-removal. At present, the technology is in various stages of bench-scale and small pilot-plant studies. The lack of a simple, low-cost technology for removing S from oil shales has greatly suppressed the commercial exploitation of oil shale resources for energy production in many regions of the world [1].

Microorganisms potentially useful in the removal of sulfur from oil shales are also relevant to coal desulfurization and mineral beneficiation processes which employ bacterial oxidation of sulfide minerals and biologically produced lixiviants for metal leaching [2]. For coal desulfurization, the emphasis has been on the microbiological oxidation of Fe-sulfides (mostly pyrite) that generally make up about 50 % of the total S content in coal, but the technology has not been commercialized.

Interested reader can find supporting and additional information regarding biological processing of coal in several review articles [3-6]. For metal leaching, commercial applications of Fe- and S-oxidizing microorganisms include dump and heap leaching processes for Cu-ores [7, 8] and biological pre-treatment of refractory Fe-sulfides for gold recovery [9-12]. A substantial amount of literature is available on biohydrometallurgical and coal-processing applications of microorganisms, whereas only few studies have addressed the biological removal of sulfur from shales.

Sulfur in Shales

Sulfur in fossil fuels exists in inorganic and organic forms. Both the inorganic and organic S-compounds occur in the reduced forms and are potential sources of SO_2 emission upon combustion. In coal and shales, the bulk of the inorganic S fraction is present as Fe-disulfides [13], mostly as pyrite (FeS_2, cubic), although marcasite (FeS_2, orthorhombic) has also been reported. Other sulfide minerals (e.g., Fe-monosulfides, ZnS, $CuFeS_2$, PbS) (Table 3) may be present in minor quantities depending on the geochemical and mineralogical composition of geological deposit. Prolonged exposure of shale and coal seams to rainwater, humidity, and air causes pyrite oxidation to elemental sulfur (S^0), thiosulfate ($S_2O_3^{2-}$), polythionates ($S_nO_6^{2-}$), and eventually to sulfates [14]. Microorganisms are largely responsible for these oxidative processes. Extensive sulfate formation due to pyrite oxidation is also a source of sulfuric acid that may have an adverse environmental impact depending on the acid-neutralization capacity of alkaline minerals (carbonates) in exposed deposits and adjacent soils and sediments.

Table 3. **Sulfide Minerals Found in Shales and Low-Grade Coals, and Various Inorganic Sulfur Compounds That Are Intermediates or Products from Biochemical Pathways of Sulfur Oxidation by *Thiobacilli***

S-compound	Chemical formula	S-compound	Chemical formula
Sulfide	S^{2-}, HS^-	Marcasite	FeS_2
Elemental sulfur	S^0	Pyrrhotite	$Fe_{1-x}S$
Thiosulfate	$S \cdot SO_3^{2-}$	Mackinawite	FeS
Tetrathionate	$^-O_3S \cdot S \cdot S \cdot SO_3^-$	Chalcopyrite	$CuFeS_2$
Trithionate	$^-O_3S \cdot S \cdot SO_3^-$	Sphalerite	ZnS
Sulfite	SO_3^{2-}	Galena	PbS
Sulfate	SO_4^{2-}	Arsenopyrite	FeAsS
Pyrite	FeS_2		

The organic sulfur fraction is complex and variable in structure. Organic sulfur compounds in fossil fuels are believed to occur as aliphatic or aromatic thiols, sulfides or disulfides, thiophenes, and their numerous derivatives [13, 15]. In shales, the main matrix of organic sulfur is kerogen, which is defined as sedimentary organic matter that is not soluble in common organic solvents. The S content may display tremendous variation within short distances even in the same seam of a coal deposit.

For oil shales from different geographical regions of the world, it has been reported that the total S content is in the range of 1.8 % to 6 % and the organic S fraction in the range of 0.1 % to 5 % [1]. Kerogens greatly vary in composition depending on the original nature of the organic matter and sedimentary environmental conditions. The organic matter in kerogens represents modified macromolecules from biopolymers and products from condensation, polymerization, and cross-linking reactions. Sulfur may incorporate into organic matter through these reactions or it may react with biopolymers or organic precursors. The eventual condensation of carbonaceous residue has also preserved organic sulfur content in kerogen.

Biotransformations of Organic Sulfur

Microbial metabolism of organic S-compounds in fossil fuels is poorly understood. Both aerobic and anaerobic transformations have been characterized with non-polar model compounds such as dibenzothiophene [16, 17], but degradative pathways for most organic S-compounds in fossil fuels remain elusive. Partial oxidation of organic S-compounds has been noted for heterotrophic bacteria such as *Pseudomonas*, *Rhodococcus*, *Corynebacterium*, *Sphingomonas*, and *Brevibacterium* spp. Condensed

thiophene structures such as dibenzothiophene are among the most recalcitrant organic S-compounds in fossil fuels. Dibenzothiophene metabolism may involve a carbon-destructive pathway [18] that leads to the formation of water-soluble metabolites and loss of carbon matrix. Sulfur-specific pathways also exist [19], with the S-entity removed from dibenzothiophene directly as SO_3^{2-} and oxidized to SO_4^{2-} without a significant loss of carbon or energy from the solid matrix of fossil fuel. Partial oxidations of condensed thiophenes to the respective sulfoxides and sulfones have been reported [20]. Naphthalene dioxygenase has been shown to be involved in the initial step of oxidative transformation of dibenzothiophene in a *Pseudomonas* sp. [21]. Dibenzothiophene utilization as the sole sulfur source by *Rhodococcus* cultures, with monohydroxybiphenyl as the transformation product, has been demonstrated [22, 23]. The amenability of organic S metabolism to coal and shale processing remains unclear. By comparison, microbial transformations of inorganic S-compounds have been elucidated more thoroughly, especially for the acidophilic Fe- and S-oxidizers that are common inhabitants of environments exposed through mining processes.

Acidophilic Fe- and S-Oxidizing Microorganisms

The microorganisms that have potential for desulfurization are capable of oxidizing Fe-sulfides, which generally make up the most of the inorganic S fraction. Fe-sulfides can serve as substrates for both Fe- and S-oxidizing bacteria. Microorganisms capable of degrading sulfide minerals and oxidizing sulfur compounds include mesophiles (optimal growth at 20-40 °C), moderate thermophiles (35-55 °C), and thermophiles (45-80 °C). Most of these microorganisms are autotrophic, i.e. they use CO_2 as a carbon source. The microbes most active in inorganic S oxidation are capable of oxidizing both the Fe- and S-entities of FeS_2 under acidic conditions (pH 1-3). The biological oxidation of pyrite and Fe^{2+} is confined to aerobic environments where O_2 is the electron acceptor. The oxidation of inorganic S-compounds also occurs under anaerobic conditions and it is coupled with Fe(III) as the electron acceptor in acidophiles. At circumneutral pH values there exist several bacteria that can couple the oxidation of S-compounds to denitrification.

Among mesophiles, *Thiobacillus ferrooxidans* derives energy for growth by the oxidation of reduced compounds of Fe and S including many different sulfide minerals.

$$4Fe^{2+} + O_2 + 4H^+ \rightarrow 4Fe^{3+} + 2H_2O$$

$$S^0 + 1.5O_2 + H_2O \rightarrow H_2SO_4$$

$$2FeS_2 + 7.5O_2 + H_2O \rightarrow 2Fe^{3+} + 4SO_4^{2-} + 2H^+$$

Ferric iron produced during oxidation is a chemical oxidizing agent of pyrite and thereby enhances the bacterially mediated oxidation. The abiotic redox reaction reduces Fe^{3+} to Fe^{2+}, which is then re-oxidized by *T. ferrooxidans*.

$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{3+} + 2SO_4^{2-} + 16H^+$$

Other acidophilic bacteria relevant in these oxidation reactions include *Thiobacillus thiooxidans* and *Leptospirillum ferrooxidans*. *T. thiooxidans* oxidizes inorganic S-compounds (including FeS_2) but not Fe, and is active at pH values as low as 0.5. *L. ferrooxidans* oxidizes only inorganic Fe(II)-compounds. All three acidophiles are common among microorganisms in active and abandoned mine sites [24]. Factors contributing to the relative predominance of *T. ferrooxidans*, *T. thiooxidans*, and *L. ferrooxidans* in mine sites, acidic run-offs, and other environments are poorly understood. Natural acid environments are usually characterized by diverse Fe- and S-oxidizing microorganisms, and such microbial consortia have many interactions and commonly involve various heterotrophic organisms and facultative anaerobes [25, 26]. Under laboratory conditions, mixed cultures can rapidly oxidize FeS_2 but it is difficult to maintain consortia that have stable proportions of *T. ferrooxidans*, *L. ferrooxidans* and other bacteria. Mixed culture work in the area of S-removal has been largely based on empirical approaches.

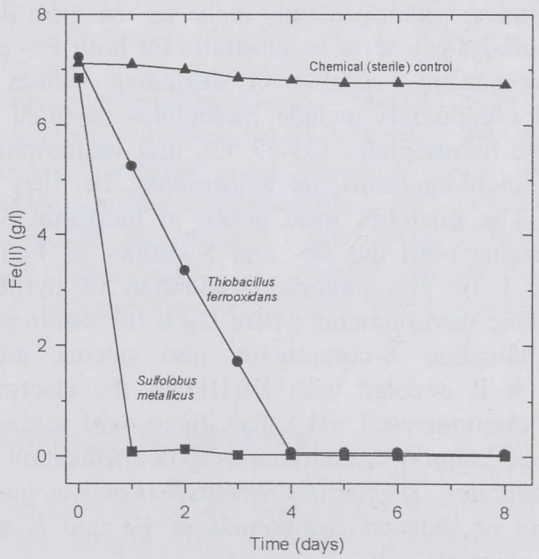

Fig. 1. Ferrous iron oxidation by *T. ferrooxidans* and *Sulfolobus metallicus* (formerly *S. acidocaldarius* strain BC). The cultures received 6.5 g Fe^{2+}/l (added as $FeSO_4 \cdot 7H_2O$) and were incubated at 22 ±2 °C (*T. ferrooxidans*) and 65 ±2 °C (*S. metallicus*). Further details can be found in [35]

Mesophilic acidophiles thrive at temperature ranges of up to about 40 °C but are inactivated at temperatures that are optimal for the moderate thermophiles. However, reaction kinetics and growth rates can be greatly improved by the use of thermophilic microbes because they can be used at elevated temperatures in the range of 40 to 80 °C. Examples of these temperature effects are illustrated in Fig. 1 that shows a comparative time course of iron oxidation by *T. ferrooxidans* (22 ± 2 °C) and *Sulfolobus acidocaldarius* (65 ± 2 °C).

Moderately thermophilic acidophiles (Table 4) have been described that are comparable to the mesophilic *Thiobacillus* and *Leptospirillum* in terms of the range of inorganic S- and Fe-compounds that they can oxidize for energy. *Thiobacillus caldus* [27] is a sulfur-oxidizer and grows best at 45-50 °C. It seems to be present in mixed cultures to afford sulfur oxidation capacity that is completely absent in *L. ferrooxidans*-like Fe-oxidizing bacteria. Some *Leptospirillum* isolates (e.g. *Leptospirillum thermoferrooxidans*) have been noted as being moderately thermophilic and capable of growing in the 40 to 50 °C range [28]. *Sulfobacillus thermosulfidooxidans* and *S. acidophilus* as well as many other gram-positive, unnamed isolates have been characterized that can oxidize several sulfide minerals [29]. Other moderate thermophiles include Fe-oxidizing isolates of *Acidimicrobium ferrooxidans* derived from *Sulfobacillus* cultures [30]. In general, there is a considerable diversity in substrate oxidation by different isolates of moderately thermophilic acidophiles. These organisms have been isolated from stable mixed cultures, suggesting cultural interactions at least at a nutritional level.

Table 4. **Examples of Microorganisms That Have Potential Capacity for the Desulfurization of Fossil Fuel**

Microorganisms	Characteristic inorganic substrate spectrum
Mesophiles (up to 45 °C)	
Thiobacillus ferrooxidans	Fe^{2+} and S-compounds, sulfide minerals
Thiobacillus thiooxidans	S-compounds, sulfide minerals
Leptospirillum ferrooxidans	Fe^{2+}, pyrite
Moderate thermophiles (35 to 55 °C)	
Thiobacillus caldus	S-compounds, sulfide minerals
Leptospirillum thermoferrooxidans	Fe^{2+}, pyrite
Sulfobacillus thermosulfidooxidans	Fe^{2+}, sulfide minerals
Sulfobacillus acidophilus	Fe^{2+}, sulfide minerals
Acidimicrobium ferrooxidans	Fe^{2+}, pyrite
Thermophiles (50-80 °C)	
Acidianus brierleyi	Fe^{2+}, S-compounds, sulfide minerals
Sulfolobus acidocaldarius	Fe^{2+}, S-compounds, sulfide minerals
Sulfolobus metallicus	S^0, sulfide minerals
Sulfurococcus yellowstonii	Fe^{2+}, sulfide minerals, S^0
Metalloshaera sedula	Fe^{2+}, sulfide minerals, S^0
Metallosharea prunae	Sulfide minerals, S^0, H_2

Thermophilic acidophiles (Table 4) assigned to the genera *Sulfolobus*, *Acidianus*, *Sulfurococcus*, and *Metallosphaera* are archaea, forming a distinct lineage from the mesophilic and moderately thermophilic acidophilic bacteria. In general, these organisms oxidize inorganic S- and Fe-compounds but the spectrum of substrates and growth requirements vary with the isolates [29]. *Sulfolobus acidocaldarius* and *S. metallicus* (formerly *S. acidocaldarius* strain BC, as well as *Acidianus brierleyi* (formerly *Sulfolobus brierleyi*), have been isolated from hot springs and coal spoils and grow at around 70 °C. Several *Sulfurococcus* isolates originate from hot springs [31]. *Metallosphaera sedula* and *M. prunae* have optimum temperatures in the range of 70 to 75 °C and grow at >80 °C [32, 33]. The thermoacidophiles have great potential in reactor leaching processes because of the favorable, rapid kinetics of bacterial growth at elevated temperatures.

Larsson et al. [4, 34] tested several thermophilic archaea for the ability to oxidize pyrite in coal. The most promising results of S-removal from coal were obtained with *Acidianus brierleyi*. There was circumstantial evidence that this organism also enhanced the removal of organic S-compounds, but the biological mechanism remains completely unknown [34]. *A. brierleyi* was not adversely influenced by organic compounds leached from coal, whereas some strains of *S. acidocaldarius* and *S. solfataricus* were sensitive to compounds leached from coal. The chemical nature of these water-soluble organic compounds was not explored further. Such findings are coal-specific and may not be reproducible with samples from other sources.

Tasa and Lindström [35] found that about 95 % of the total S-content of a finely ground oil shale sample was removed in *Sulfolobus acidocaldarius* cultures within four days and there was no evidence for inhibitory effects due to leachates. Figure 1 shows a comparison of ferrous iron oxidation by mesophilic *Thiobacillus ferrooxidans* and thermophilic *Sulfolobus acidocaldarius* cultures. In this experiment, iron was oxidized within 24 hours in the thermophilic culture whereas it took four days for *Thiobacillus ferrooxidans*. Similarly, the leaching of pyrite from oil shales was fast with *Sulfolobus acidocaldarius* compared with the mesophilic *T. ferrooxidans*. In bioreactor experiments, *Sulfolobus acidocaldarius* removed 94 % pyrite from finely ground oil shale sample within 4 days, contrasted with 67 % removal of pyrite by *T. ferrooxidans* within 11 days [35].

Leaching processes involving thermophilic bacteria are considered particularly promising for the biological leaching of sulfide minerals such as chalcopyrite and pyrite that tend to require long contact times in mesophilic bacterial processes. An added advantage in thermophilic leaching processes is the heat generation from exothermic oxidation reactions. The generation of heat can be so intensive with bacterial oxidation of sulfide concentrates that a cooling system would be required for mesophilic bacteria in order to prevent prohibitively high temperatures.

With thermophilic bacteria, the heat generation would help maintain the elevated temperature range.

Environmental Factors

The kinetics and efficiency of biological removal of S from fossil fuels are a complex function of many interactive factors. These include the metabolic capabilities of microorganisms involved, temperature, acidity, pO_2, pCO_2, nutrients (e.g., P, N, microelements), toxic compounds, mass transfer rates, mineralogy, chemical composition of fossil fuel, and specific surface area of solids. Many of these factors have been discussed in reviews that focus on the bacterial leaching of metals from sulfide ores [8, 10, 24]. Although the solid phase composition in sulfide ores is different from oil shales, the kinetic and thermodynamic principles governing the oxidation processes for Fe and S-compounds are applicable to both ores and shales. Testing of experimental variables is extremely crucial in developing information that can eventually be used for optimization and kinetic modeling.

Most of the bacteria able to remove sulfuric compounds from fossil fuels have the optimum pH between 1.5-3. The pH has a major influence on the biological and chemical leaching rates. Acid attack becomes increasingly more aggressive at low pH values, but the chemical stability of Fe^{2+} is increasingly improved. *T. thiooxidans* and *L. ferrooxidans* grow at pH values at least as low as pH 0.5. Acidophilic thiobacilli tolerate near circumneutral pH values but, in practice, at pH values above 3 the chemical oxidation of ferrous iron becomes increasingly faster and the product, ferric iron, precipitates and does not participate in the leaching reactions.

The suitability of sulfide-containing materials for bacterial oxidation can be assessed on the basis of acid-generating (mostly FeS_2) and acid-consuming (mostly carbonates) minerals. Brierley and Brierley [36] have described the biological acid production potential, based on the estimation of acid generation, acid generation potential, and acid neutralization potential.

Estonian oil shales contain organic, sandy-clay and carbonate constituents. The carbonate fraction mostly comprises calcite [37]. Acid-consumption due to carbonate dissolution leads to increasing pH values and precipitation of $CaSO_4$, and this is counteractive to the bacterial leaching.

$$CaCO_3 + 2H^+ + SO_4^{2-} + H_2O \rightarrow CaSO_4 \cdot 2H_2O + CO_2$$

It is possible to deal with excessive carbonate reaction by titration with H_2SO_4 to satisfy the acid consumption in initial experiments, but the

gypsum ($CaSO_4 \cdot 2H_2O$) thus formed covers mineral surfaces and acts as barrier to the diffusion of reactants and products.

The precipitation of ferric iron occurs in the form of hydroxides, jarosites, and hydroxysulfates that also decrease diffusion rates because they tend to coat mineral surfaces. Jarosite precipitation ($XFe_3(SO_4)_2(OH)_6$, where $X = Na^+$, K^+, NH_4^+, or H_3O^+) is considered to be the most important reaction controlling ferric iron solubility at pH <3 [38]. At higher pH values other solid phases such as schwertmannite ($Fe_8O_8(OH)_6SO_4$), ferrihydrite ($Fe_5HO_8 \cdot 4H_2O$), and goethite (α-FeOOH) are more dominant [39, 40].

Silicate minerals found in shales and coal are subjects to weathering reactions in acid solutions, mediated by proton attack. Proton attack is enhanced at low pH values and can liberate structural monovalent cations from mica interlayer position in solution [41]. Depending on the pH, monovalent cations from mica weathering may become incorporated into jarosites, which thereby act as sinks for monovalent cations. Silicates vary in their susceptibility to weathering processes. Microbiological weathering of feldspars and micas has been reported [42-44] but the actual rates are highly variable depending on the mineral and experimental conditions. Typical products of mica weathering in acid solutions relevant to biological desulfurization processes are expandable layer silicate phases (e.g., vermiculite) and mixed layer mica/vermiculite structures.

The prevailing temperatures at mine sites show a great deal of variation. In underground mines the temperatures vary relatively little on a seasonal basis and are usually around 10-15 °C depending on geological conditions. In surface heaps, waste piles, and coal spoils, temperatures may be elevated depending on the amount of Fe-sulfides present in the pile. Elevated temperatures that are prohibitively high to mesophilic bacteria are not uncommon in coal spoils. Mesophilic Fe- and S-oxidizers are found active at near-zero temperatures, but the growth and metabolic activity are slow and the minimum temperature still supporting growth remains poorly defined.

As with all biological reactions, the rate of iron and sulfur oxidation is highly dependent on the temperature. Decrease in the temperature below the optimum is accompanied with a decline in the rate of substrate oxidation and an increase in the generation time. Different strains of the same species of bacteria may have slightly different temperature optima. With *T. ferrooxidans*, optimum temperatures reported in the literature range mostly from 25 to 35 °C. The maximum permissive temperatures for mesophilic thiobacilli are around 40-44 °C [45] but considerable strain-dependent variation is likely to exist.

Ahonen and Tuovinen [46, 47] reported that the temperature quotient Q_{10} for iron and sulfur oxidation was about 2. The Q_{10} is defined as the increase in the rate or rate constant of the reaction for each 10 °C in the temperature. The activation energies (E_a) for iron and sulfur oxidation by

T. ferrooxidans were 80 kJ mol^{-1} and 65 kJ mol^{-1}, respectively. The effect of temperature in biological leaching systems is complex because of the formation of reaction zones that become diffusion barriers and decrease the temperature-dependence of the reaction. While elevated temperatures up to 70-80 °C are selective for thermophilic archaea (*Sulfolobus*, *Acidianus*, *Metallosphaera*), they also increase the rates of chemical reactions and decrease the solubility of O_2 and CO_2.

Acid leaching systems contain numerous water-soluble constituents that are potentially toxic or inhibitory to biological processes. These toxicity effects may be due to inorganic ions (metal ions) or organic compounds leached from the fossil fuel matrix. The toxicity varies with the bacterial strain, environmental conditions, the length of exposure, and the concentration of the element. Figure 2 summarizes the range of metal concentrations inhibitory to acidophilic Fe- and S-oxidizing bacteria. The data in Fig. 2 are all based on $FeSO_4$ oxidation data pooled from published studies, with variations in test strains, experimental conditions, and assay methodology.

By comparison with heterotrophic bacteria living in circumneutral pH values, acidophilic thiobacilli can tolerate relatively high concentrations of Cu, Co, Ni, and Zn in their growth medium. This property is one of the underlying requisites for their use in metal leaching processes.

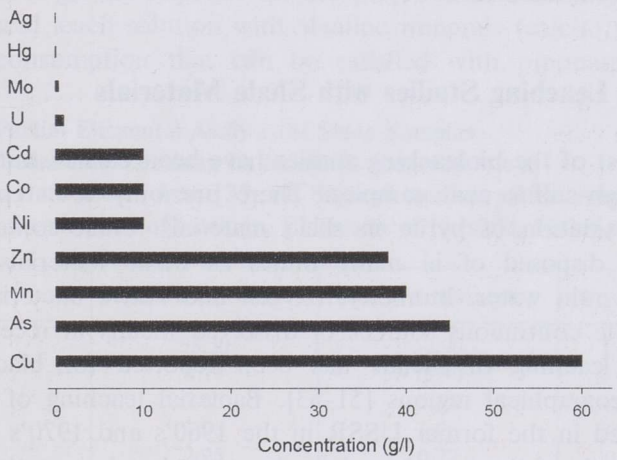

Fig. 2. Spectrum of resistance of *Thiobacillus ferrooxidans* to different metals. The columns indicate the concentration ranges where activity or growth of *T. ferrooxidans* has been reported. Data have been pooled from several literature sources that have considerable differences in experimental variables and methodology of establishing the level of toxicity or resistance. Substantial variation occurs with different strains. The experimental design has not included testing for complexation, precipitation, or redox speciation. These data can be constructed as a relative ranking of metal toxicity based of magnitude in the inhibitory concentration

The biochemical basis of this high level of resistance is not known. Silver, mercury, and molybdenum are among the most toxic metals to Fe- and S-oxidizers. Specific mechanisms of toxicity and resistance are mostly unknown. Biochemical basis of resistance to toxic metals is known in the case of mercury and it involves a mercuric reductase enzyme complex that reduces Hg^{2+} to Hg^0 [48]. Elemental mercury thus formed is removed from the cell and growth medium because of its volatility. The toxicity of metals to bacteria is subject to their bioavailability which can be altered with chelating agents; e.g., yeast extract can decrease the effective, bioavailable concentration of metals because of a complexation effect. In the presence of clay minerals (e.g., kaolinite and illite) and oxides (Al_2O_3), bacteria can be partially protected owing to cation exchange properties of clays that decrease the available concentrations of metals.

Some Estonian shales contain toxic elements such as uranium, molybdenum, vanadium at levels [49] that are clearly toxic to bacteria if completely solubilized in leach solution. The geochemical reactions controlling the solubility of elements such as molybdenum and vanadium are poorly understood in biological leaching systems. Factors influencing the bioavailability of these and other elements in the presence of bacteria and mineral sulfide substrates have yet to be elucidated. Inhibitory effects attributed to the leaching of toxic elements into aqueous phase have been reported in shale leaching experiments [50, 51] but the toxic constituents have not been identified.

Biological Leaching Studies with Shale Materials

By far, most of the bioleaching studies have been carried out with sulfide ores or high-sulfur coal samples. There are only few reports on the bacterial oxidation of pyrite in shale materials. Shale-containing seams have been disposed of in many mines as waste materials and, when exposed to rain water, humidity, oxygen and native bacteria over time, they become continuous sources of dissolved metals in receiving waters. Biological leaching of metals has been reported for black shales in different geographical regions [51-53]. Bacterial leaching of black shales was explored in the former USSR in the 1960's and 1970's [52], but no specific details of these projects have been disclosed since that time. The underlying interest of the Soviet research program was related to uranium processing.

Shales are composed of silicate phases and organic matter, and bacterial leaching of shales is of interest in assessing the potential mobility (and recovery) of heavy metals [51, 53]. The solubilization of pyrite from high-sulfur shales is another reason for these studies because it is the potential source of sulfuric acid [50]. Bacterial oxidation can also be viewed essentially as a pretreatment step in preparation of kerogen

concentrates [54, 55]. There are also pressing issues of environmental pollution that can stem from exposed shales in waste heaps and dumps [35]. As indicated in Table 5, Estonian shale samples contain toxic elements (U, Mo, V) that can potentially be solubilized when shales are exposed to acid conditions and pyrite inclusions oxidized to water-soluble products.

Bacterial leaching of U, V, Mo, Zn, and Pb from Polish shale samples was investigated in long term (7 months) experiments by Sztaba et al. [56], Konopka and Sztaba [57], and Konopka et al. [53]. The bacterial leaching of zinc and lead was insignificant, as most of the Zn and Pb content (95 % Zn and 55 % Pb) was leached chemically during the initial stage of the experiments. Maximum yields of dissolution in these experiments were 75 % U, 60 % Mo, and 47 % V.

Short-term shake flask studies with Estonian black shale samples have also suggested that Fe- and S-oxidizing bacteria enhance the dissolution of these elements [51, 58]. This enhancement may be due to co-solubilization with pyrite dissolution, or increased acid dissolution via bacterial production of sulfuric acid. Some shales are pyrite-rich or they have pyrite-containing zones interlayered with shales. Shale samples have been tested for bacterial leaching that have contained as much as 11 % Fe as pyrite [50].

While pyrite inclusions in shales are biologically degradable, the depyritization process greatly depends on the presence of accessory minerals. Reaction of acid leach solution with alkaline minerals (calcite, dolomite) causes acid consumption that can be satisfied with pretreatment with

Table 5. **Partial Elemental Analysis of Shale Samples Used in Preliminary Leaching Experiments (concentration, % wt/wt). Data pooled from [35, 51, 58] and courtesy of Dr. Antti Vuorinen, Department of Geology, University of Helsinki, Finland**

Component	Black shale (Toolse)	Black shale (Maardu)	Oil shale
SiO_2	55.30	52.90	1.60
Al_2O_3	8.18	13.40	3.05
Fe_2O_3	7.85	5.15	4.15
MgO	1.08	1.00	3.23
CaO	2.95	0.22	30.00
Na_2O	0.82	0.80	0.04
K_2O	4.92	8.30	1.00
MnO	0.04	0.02	NA*
Ti_2O	0.51	0.83	0.18
S	2.21	1.47	4.75
U	0.0095	0.0026	NA
Mo	0.0245	0.0013	NA
V	0.0740	0.0240	NA
Loss of ignitation	18.00	17.90	56.00

* NA, not analyzed.

sulfuric acid [35, 59]. As the high CaO content indicates (Ida-Virumaa oil shale sample, Table 5), some materials contain Ca carbonates that cause excessively high acid consumption when subjected to acid leaching conditions. Biological oxidation of supplemental S^0 has been proposed as a source of sulfuric acid to compensate for acid consumption [54].

Vrvic et al. [55] reported that up to 95 % of the sulfur content was removed from oil shale samples (from Aleksinac, former Yugoslavia) within a week by exposing the finely ground oil shale to the leaching action of *T. ferrooxidans*. In a study conducted with an Indian black shale sample, Mahapatra et al. [50] achieved >95 % removal of pyrite after three weeks of leaching in *T. ferrooxidans* cultures. For Estonian oil shales (from Ida-Virumaa), Tasa and Lindström [35] reported 90 to 96 % removal of pyrite (5 % pulp density) within a week, based on the oxidative action of *T. ferrooxidans* or *Sulfolobus acidocaldarius* in shake flask experiments. In stirred tank bioreactors that allow for improved process control, *T. ferrooxidans* oxidized about 65 % of the pyrite content in 10 % pulp density to water-soluble products in 11 days, while *Sulfolobus acidocaldarius* removed 94 % of the pyrite content in four days. These comparisons clearly underscore the rate and other kinetic data in favour of the thermophilic *Sulfolobus* species.

Concluding Remarks

Chemical depyritization of oil shales often leads to substantial alterations in the organic matter fraction (kerogen) and a loss of BTU content in shales. Biologically mediated oxidative removal of pyrite from oil shale is the least non-destructive method. Biological removal can also be used for non-destructive purification of kerogen for structural studies. The complexity of shales due to inert mineral phases (quartz), clay minerals with surface active properties, organic matter fraction particles, oxidizable sulfide minerals such as pyrite, and acid-reactive carbonates poses a challenge to identify reaction mechanisms, pathways, and products. Some shales contain potentially toxic metals but their solubility, bioavailability, and geochemical speciation are unknown at this time.

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